COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

COT STATEMENT ON TRYPTOPHAN AND THE EOSINOPHILIA-MYALGIA SYNDROME

Introduction

1. In the autumn of 1989, a previously unrecognised epidemic illness was reported in the United States. Its principal features included an elevated eosinophil count and severe myalgia, often accompanied by arthralgia, oedema, dyspnoea, fever and rash, and it was termed the eosinophilia-myalgia syndrome (EMS). The syndrome was quickly associated with the consumption of L-tryptophan-containing dietary supplements and these were withdrawn from the US market. In total, over 1500 cases of EMS were reported in the US, including at least 37 deaths.

2. Several cases of EMS were also reported in the UK, Germany, Canada, Belgium, France, Israel and Japan. In 1990 the COT endorsed a ban on the addition of tryptophan to foods (including supplements) in the UK. Since that time, a number of epidemiological, analytical and toxicological studies have been published, which have investigated possible causes of the EMS epidemic. It has been claimed that the causes of EMS are now known and that there is no need for the continuing ban of added tryptophan in food[1]. The Committee was therefore asked to consider the available data on tryptophan and EMS and to advise on the risks to health of tryptophan in food supplements.

Background

3. L-tryptophan is one of 8 essential amino acids that cannot be made by the body and therefore must be included in the diet daily to maintain nitrogen balance. It is important for protein synthesis and is the precursor of a number of biologically important compounds including serotonin, melatonin, tryptamine, quinolinic acid, kyneuric acid. It is also the precursor of NAD and NADP and can replace niacin[2].

4. Good food sources of L-tryptophan are foods high in protein such as meat (230 mg/100g in beef), cheese (325 mg/100g in Edam cheese), fish, milk and eggs (165 mg/100g)[2]. In 1991, the Committee on Medical Aspects of Food and Nutrition Policy (COMA) concluded that the protein quality of the UK diet was sufficient not to set recommendations for individual amino acids. It

noted that studies of the amino acid pattern of the British diet indicated that the average household consumes a diet which supplies adequate amounts of essential amino acids[3]. There have been no recent estimates of dietary L-tryptophan intakes but the National Food Survey of 1974 indicated the average intake to be 890 mg/day (equivalent to 14.8 mg/kg bw/day in a 60 kg adult)[4]. Tryptophan requirements in adults have been estimated to be 6 mg/kg bw/day[5].

5. Although the addition of isolated tryptophan to foods in the UK is generally banned, the addition of tryptophan is permitted in certain foods such as infant formulas, follow on formulas and processed cereal based foods and baby foods, which are regulated by EU legislation. Tryptophan was permitted in such foods to ensure protein quality and it was understood that natural protein sources of tryptophan, rather than isolated tryptophan, were used. EU legislation further permits the addition of tryptophan to foods for particular nutritional uses (PARNUTS). Manufacturers of certain categories of PARNUTS food such as those intended for sportsmen, dieting or diabetics are obliged to notify the FSA prior to marketing.

6. Prior to the 1990 ban, L-tryptophan-containing dietary supplements were typically used for insomnia, depression and premenstrual syndrome, and had been used by bodybuilders. In the UK, one to two 500 mg tablets were typically taken per day, usually at night. In the US, at the time of the EMS outbreak it was reported that people were taking up to 8000 mg/day supplemental L-tryptophan.

Eosinophilia-myalgia syndrome

7. The case definition of EMS by the US Centers for Disease Control (CDC) was: (1) a total eosinophil count greater than 1×10^9/L, (2) generalised myalgias at some point during the course of the illness of sufficient severity to limit the ability to pursue normal activities, and (3) exclusion of other neoplastic or infectious conditions that could account for the syndrome[6]. Case reports also described arthralgia, swelling of the extremities, rash, fever, cough, interstitial lung disease, arrythmias, ascending polyneuropathy and sclerodermiform skin thickening in EMS patients. After ceasing to take L-tryptophan supplements, improvement was slow, with symptoms continuing to progress in some patients[7]. While most symptoms were reported to have improved 2 years later, a study of 205 patients reported that cognitive changes (impaired memory, impaired concentration or mood change, reported in 28% of patients) and neuropathy had not[8].

8. The features of EMS closely resemble the 1981 Spanish toxic oil syndrome epidemic. The toxic oil syndrome was associated with the consumption of adulterated rapeseed oil, affected approximately 20,000 people and resulted in more than 300 deaths; however, the causes were never established. The features of toxic oil syndrome included fever, rash, pneumonitis, myalgias, eosinophilia, neuromuscular abnormalities, pulmonary

hypertension and scleroderma-like skin changes. The differences between the two syndromes are few, the incidence and intensity of pulmonary effects such as coughs, infiltrates, dyspnoea and plural effusions being fewer and less severe in EMS patients than toxic oil syndrome patients[7].

9. There are also some similarities with eosinophilic fasciitis, a rare disorder first described in 1975, which is characterised by eosinophilia of both the peripheral blood and connective tissue, fascial inflammation and thickening, and pain and swelling of the extremities[9,10]. The pathophysiology and aetiology of eosinophilic fasciitis remain poorly understood.

Possible causes of EMS

Link to L-tryptophan produced by one manufacturer

10. L-tryptophan used in dietary supplements on sale in the US was manufactured by 6 bulk manufacturers. In epidemiological studies, 97-100% of EMS cases meeting the CDC criteria were traced to L-tryptophan manufactured by one company, Showa Denko KK of Japan[11,12,13]. Furthermore, EMS symptoms were reported to resolve in patients who switched from Showa Denko L-tryptophan to non-implicated L-tryptophan supplements[14,15]. The authors of one of the epidemiological studies noted that all the EMS cases that could be traced back to Showa Denko in their study had consumed L-tryptophan produced between October 1988 and June 1989, possibly suggesting that manufacturing variables were important.

11. National surveillance in the US indicated that on average 10% of consumers of Showa Denko EMS-implicated L-tryptophan were diagnosed with EMS and there was no dose-risk relationship[7]. EMS patients who had taken L-tryptophan supplements had taken them for between 0 and 3,668 days before onset of illness (0 indicating onset of illness on the same day as first taking L-tryptophan supplements), with a median of 127 days and a mean of 275 days[6].

Identification of contaminants in EMS-implicated L-tryptophan

12. Showa Denko L-tryptophan was produced by a fermentation process involving the use of *Bacillus amyloliquefaciens*. On 25 December 1988 the company started using a new strain (Strain V) of *Bacillus amyloliquefaciens* which increased the synthesis of two intermediates in L-tryptophan biosynthesis, serine and 5-phosphoribosyl-1-pyrophosphate. Additionally, the amount of powdered activated carbon used in purification steps was reduced from usually 20 or more kg/batch in 1988 to 10 kg in most batches in 1989. Furthermore, between October 1988 and June 1989 some L-tryptophan batches partially bypassed a filtration step using a reverse osmosis filter to remove chemicals with molecular weights of more than 1000. Statistical analysis showed significant associations between batches of L-tryptophan associated with EMS and the use of Strain V *Bacillus amyloliquefaciens* and

the reduction in the amount of activated carbon used[11].

13. More than 60 minor contaminants were identified in EMS-associated batches of Showa Denko L-tryptophan. On comparing batches of EMS-associated Showa Denko L-tryptophan, non-EMS associated Showa-Denko L-tryptophan and control L-tryptophan from another manufacturer, six of these contaminants were associated with EMS[17]. Three of the contaminants were identified as 1,1'-ethylidenebis[tryptophan] (EBT), 3-(phenylamino)-L-alanine and 2(3-indolylmethyl)-L-tryptophan. Two others were later identified as 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo-[2,3-b]-indole-2-carboxylic acid and 2-(2-hydroxyindoline)-tryptophan[17]. One of the contaminants remains unidentified.

Toxicological studies of tryptophan and its implicated contaminants

14. L-tryptophan has low oral toxicity[2]. The lowest reported LD_{50} is 1.6 g/kg bw in rats, where death was thought to have resulted from an accumulation of metabolites such as ammonia and urea. Equivocal changes in the liver, including fatty change and fibrosis, were seen in a study in rats dosed with 250 mg/kg bw/day for 3 days[18]. In a rat carcinogenicity bioassay conducted by the US National Cancer Institute no evidence of carcinogenicity was found[2].

15. Treatment of rats with adrenal dysfunction with 100 or 1000 mg/kg bw/day L-tryptophan (Japanese pharmacopoeia grade, not manufactured by fermentation) by gavage for 7 days resulted in a statistically significant increase in the number of eosinophils in the peripheral blood[19]. Guinea pigs supplemented orally via gelatine capsules with 400 mg/kg bw/day L-tryptophan (purchased from a laboratory chemical supplier several years after Showa-Denko had ceased production of L-tryptophan) showed a statistically significant decrease in the number of circulating eosinophils and increase in the number of eosinophils in the bronchial alveolar lavage fluid compared to controls[20]. The significance of these results is not clear.

16. An investigation in female Lewis rats, dosed by gavage for 38 days with 1600 mg/kg bw/day EMS-implicated L-tryptophan, showed fasciitis and perimyositis, whereas those dosed with 1600 mg/kg bw/day control L-tryptophan did not[21]. A study was conducted to determine whether EBT was responsible for these effects. All animals treated with EMS-associated L-tryptophan (2000 mg/kg bw/day), EBT (40 mg/kg bw/day) or EBT plus control L-tryptophan by gavage, 6 days/week for 6 weeks showed significant myofascial thickening compared to animals receiving control L-tryptophan or vehicle-only controls[22]. However, even animals treated only with control L-tryptophan showed a mild but statistically significant increase in the thickness of the myofascia compared to vehicle-only controls. This thickening of the myofascia could be related to prior resolved inflammation rather than a direct effect of the tryptophan. Immune effects, including increased frequency of CD8, Ia and IL-2 receptor-positive cells in the peripheral blood, were observed in EMS-associated L-tryptophan-treated animals only. Control L-tryptophan in both studies was US pharmacopoeia-grade L-tryptophan which had not been

associated with cases of EMS and contained no measurable EBT.

17. In a study in mice, administration of EBT (40 µg/kg bw/day, i.p. for up to 6 weeks) resulted in an inflammatory reaction in the dermis and subcutis, with hyperplasia of the epidermis in 20% of animals[23]. Focal inflammation was also seen in saline and L-tryptophan (non-EMS implicated, 30 mg/kg bw/day)-treated animals but was not as severe as in EBT-treated animals. Cellular infiltration was accompanied by fibrosis and destruction of the adipose layers and panniculus carnosus muscle. These changes were more severe in EBT-treated animals than in other groups.

18. Three female Lewis rats were treated with 40 mg/kg bw/day EBT and four with 40 mg/kg bw/day non-EMS-implicated L-tryptophan, i.p., for up to 132 days, with tail vein blood drawn at days 26 and 72 for haematological analysis[24]. No abnormalities were seen in the four L-tryptophan-treated rats. Some necrotic muscle fibres were observed in all three animals treated with EBT and in two there was inflammation of the fascia and perimysium. No abnormalities were observed in the four L-tryptophan-treated rats.

19. *In vitro* studies have shown that EBT stimulates human fibroblast proliferation and increases collagen synthesis and type I collagen mRNA levels in human fibroblasts[25,26].

20. 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA), the breakdown product of EBT, has been shown to affect the survival of 1 month old spinal cord cultures derived from fetal mice. This effect only occurred with the S-isomer, and not the R-isomer of the compound. Co-treatment of cultures with antisera to interleukin-1α or the murine IL-1 receptor abolished the effect[27].

21. Mononuclear cell supernatants from hypereosinophilic syndrome patients (an idiopathic disease in which eosinophil levels in the peripheral blood are raised to > 1500/µL for 6 months or more, leading to multiple organ system effects), when incubated with IL-2 and either EBT or MTCA, supported the formation of colonies with neutrophils, macrophages and 'a small number' of eosinophils[28]. Two to three-fold increased levels of IL-6 were found in the supernatant of mononuclear cells from a hypereosinophilic syndrome patient and two normal volunteers when stimulated with IL-2 and either EBT or MTCA. IL-5 has been reported to be induced by stimulating human splenic T cells *in vitro* with EBT[29].

22. 3-(Phenylamino)-L-alanine is closely related to aniline contaminants identified in the oil consumed by patients of the toxic oil syndrome. One of the toxic oil syndrome contaminants, 3-(phenylamino)-1,2-propanediol, is metabolised to 3-(phenylamino)-L-alanine by rat hepatocytes and human liver tissue[30]. However, in a subchronic toxicity study of 3-(phenylamino)-L-alanine in rats, no EMS-like or other abnormalities were found[31]. One study showed that 3-(phenylamino)-L-alanine affects the binding of L-tryptophan to rat hepatic nuclear membranes (which contain a tryptophan receptor) *in vitro*[32]

but the significance of this is not clear.

23. None of the other four contaminants appear to have been studied toxicologically. Two were identified only relatively recently[17] one remains unidentified.

Susceptible subgroups

24. One study showed that people with EMS were more likely to have a genotype associated with poor CYP2D6-dependent metabolism[33]. However, the majority of EMS cases had extensive CYP2D6-dependent metabolism.

EMS not linked to implicated L-tryptophan

25. A small number of EMS cases were not traced to Showa Denko L-tryptophan. In two of the epidemiological studies, all but one EMS case in each study was conclusively linked with Showa Denko L-tryptophan[13,14]. In one of the studies, the authors analysed the L-tryptophan consumed by the non-Showa Denko associated case and other L-tryptophan supplements. They observed that the L-tryptophan consumed by the non-Showa Denko associated case contained EBT and that the HPLC 'signature' exactly matched that of other Showa-Denko L-tryptophan samples and did not match that of L-tryptophan samples from the manufacturer it was apparently from. The authors suggested that incorrect labelling at some stage of the tableting or distribution process could have lead to erroneous information on the source of the L-tryptophan[13].

26. One of the epidemiological studies also looked at 'possible' EMS cases, which did not meet the CDC criteria for EMS but in which there was either raised eosinophil count in the absence of other symptoms or 2 or more other features of EMS without raised eosinophil count[13]. Thirty-two possible EMS cases were identified from users of non-Showa Denko L-tryptophan.

27. Twelve case of EMS were reported to have been diagnosed in Canada between July 1992 and June 1993, more than 2 years after the EMS epidemic[34]. The cases were not linked to L-tryptophan consumption. The authors also identified 14 'possible' EMS cases, which did not fully meet the CDC criteria for EMS but noted that there were other possible diagnoses for these cases, including eosinophilic fasciitis, hypereosinophilic syndrome, Churg-Strauss syndrome (severe asthma associated with raised eosinophil counts), small vessel vasculitis, fibromyalgia, atypical arthritis, drug reactions and asthma.

28. Three percent of 1056 cases of EMS in the USA denied having taken L-tryptophan supplements[6]. In addition, of 1055 cases for whom the information was available, one had first reported symptoms in 1954, one in 1980, one in 1981, four in 1983, two in 1985, four in 1986, 13 in 1987, 31 in 1988 and 31 in 1989 before May, i.e. prior to the EMS epidemic. It is not clear

whether these cases had consumed L-tryptophan supplements.

29. Two case reports are available of cases of EMS in people who denied having taken L-tryptophan supplements[35,36].

30. Several reports have described cases of EMS and EMS like symptoms linked to consumption of 5-hydroxy-L-tryptophan, a metabolite of L-tryptophan[37,38,39]. 5-Hydroxy-L-tryptophan is not manufactured in the same way as L-tryptophan, but is either synthesised from 5-benzyloxyindole or extracted from the seeds of the African tree *Griffonia simplicifolia*. A contaminant, 1,2,3,4,4a,9a-hexahydro-β-carboline-3-carboxylic acid, which has a similar chemical structure to MTCA, has been found in 5-hydroxy-L-tryptophan produced by both methods[39,40].

Current availability of tryptophan medicines and supplements

31. Due to its role as a precursor to serotonin, L-tryptophan is available in the UK as a prescription drug for the treatment of depression. Its use is limited to severe and disabling depression which has continued for more than 2 years, following the trial of other antidepressant drugs and only together with the use of other antidepressant drugs. The dose taken is 1 to 2 grams, three times a day (maximum dose 6 grams), to be reduced if the patient is elderly or taking monoamine oxidase (MAO) inhibitors. Prescribing GPs are advised to regularly survey and monitor eosinophil count, haematological changes and muscle symptomatology.

32. Only one product is currently available in the UK, the Merck Pharmaceuticals product Optimax. At the time of the EMS epidemic four possible cases of EMS were linked to Optimax. One of the cases was confirmed as matching all the CDC criteria for EMS[41]. It has been suggested that Optimax may not have been the only source of L-tryptophan this case had been exposed to[42]. Because of the concern over EMS, a unit was established by the manufacturers in 1994 to facilitate the monitoring of patients prescribed Optimax. Prescribers and patients must be registered with the unit in order to receive Optimax. Prescribers are sent an initial questionnaire on registration, followed by questionnaires at 3 and 6 months and thereafter every 6 months. Since monitoring began over 5000 patients have been treated with Optimax, with a mean dose of 2228 mg/day L-tryptophan[42]. The manufacturers stated that up-to-date questionnaires are available for 96% of patients. No patients meeting all the CDC criteria for EMS have been identified.

33. The purity criteria for the L-tryptophan used in Optimax are stated to be similar to the European Pharmacopoeia monograph[42]. In particular there is a limit for EBT of 10 ppm.

34. A small number of dietary supplements containing L-tryptophan are currently available in Belgium and the Netherlands[1,43] and one product is

available in the Czech Republic[44]. Literature searches have not identified any reports of cases of EMS in these countries. The source of the L-tryptophan used in these products and the purity criteria used in their manufacture are not clear. The Health Council of the Netherlands has recommended a maximum supplementary intake of 0.6 g/day tryptophan. This was based on the amount of tryptophan that would be supplied by the protein intake advised for women in the Netherlands. No specific purity criteria were recommended[45].

COT evaluation

35. On the balance of evidence, it is likely that L-tryptophan *per se* was not causal for EMS, and that EMS was due to one or more contaminants. Changes to the manufacturing and purification process by one particular manufacturer may have increased levels of these contaminants to harmful levels. EBT was found in some samples of non-EMS implicated tryptophan supplied by other manufacturers, although at lower levels.

36. However, there are some uncertainties. These include whether EMS could arise sporadically. There are no reliable data on the prevalence of EMS and as EMS is a difficult condition to define, with a variety of non-specific symptoms, it is possible that mild cases may not be diagnosed and that there could have been underreporting of EMS prior to its recognition in 1989. An epidemiological study indicated that the use of L-tryptophan supplements increased substantially between 1988 and 1989, the start of the EMS epidemic. It therefore cannot entirely be ruled out that the apparent epidemic may have been due to the increased use of L-tryptophan supplements and the recognition of EMS.

37. There is little information on the possible mechanisms that could lead to EMS. The limited available animal data have not reproduced all the features of EMS. Administration to rats of EMS-implicated L-tryptophan and the contaminant EBT resulted in myofascial thickening. A US pharmacopoeia-grade L-tryptophan, which was not associated with cases of EMS and did not contain any EBT, also caused increased myofascial thickening but to a lesser degree. This thickening of the myofascia could be related to prior resolved inflammation rather than a direct effect of the tryptophan. The toxicological significance of this is therefore unclear.

38. It is not possible to determine specific contaminants causal of EMS. EBT has reproduced some of the features of EMS, such as fasciitis, in animals, but not others, such as eosinophilia. The presence of EBT could be a marker for another contaminant which is responsible for EMS or it could be a number of contaminants that are responsible for the features of EMS. Some of the contaminants found in EMS-implicated L-tryptophan have not been studied toxicologically.

39. It has not been possible to identify any particular subgroups with increased susceptibility to developing EMS.

40. L-Tryptophan supplements are on sale in a small number of countries, including the Netherlands, Belgium and the Czech republic. We are not aware of any cases of EMS being reported in these countries; however, it is not clear that symptoms of EMS would be monitored for or necessarily recognised. We have not been informed of any specific purity criteria used in the manufacture of these supplements.

41. L-Tryptophan is also available on prescription as a licensed medicine, the Merck product Optimax. GPs are advised to closely monitor patients receiving Optimax, including regularly monitoring eosinophil count, and are sent questionnaires at 6 monthly intervals to complete and return. Since Optimax returned to the market in 1994 over 5000 patients have been prescribed Optimax, with no confirmed cases of EMS. This provides reassurance that the purity criteria used for Optimax reduces the risk of EMS. The purity criteria for the L-tryptophan used in Optimax are stated to be similar to the European Pharmacopoeia monograph for L-tryptophan, and include a limit for the contaminant EBT of 10 ppm.

Conclusions

42. We conclude that the new data offer reassurance that as a prescription medicine, tryptophan has not resulted in a detectable increase in risk of EMS. Applying an uncertainty factor of 10 to the mean therapeutic dose of 2228 mg tryptophan per day, to allow for uncertainty with respect to the actual cause of EMS, indicates that a dose of 220 mg tryptophan per day as a dietary supplement would not present an appreciable risk to health, providing that it meets the purity criteria specified in the European Pharmacopoeia.

COT Statement 2004/01
June 2004

Addendum

In December 2005, the COT agreed that the statement should be amended to state that the mean therapeutic dose referred to in the conclusion was without adverse effect and so represented a NOAEL**.**

References

1. Heaton S (2002) The case for the removal of the ban on tryptophan as a food supplement. Report commissioned by the Institute for Optimum Nutrition to the UK Food Standards Agency regarding the removal of the ban on L-tryptophan in food supplements specified in the Tryptophan in Foods Regulations 1990

2. Sainio E-L, Pulkki K, Young SN (1996) L-tryptophan: biochemical, nutritional and pharmacological aspects. *Amino Acids* **10**:21-47

3. COMA (1991) Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Committee on Medical Aspects of Food and Nutrition Policy. The Stationery Office, London.

4. Buss DH, Ruck NF (1977) The amino acid pattern of the British diet. *J. Hum. Nutr.* **31**:165-169

5. WHO (1991) Update of Technical Report Series No. 724. Energy and Protein Requirements: Report of a Joint FAO/WHO/UNU Expert Consultation

6. Swygert LA, Maes EF, Sewell LE, Miller L, Falk H, Kilbourne EM (1990) Eosinophilia-myalgia syndrome: results of national surveillance. *JAMA* **264**:1698-1703

7. Hertzman PA, Falk H, Kilbourne EM, Page S, Shulman L (1991) The Eosinophilia-Myalgia Syndrome: the Los Alamos conference. *J. Rheumatol.* **18**:867-873

8. Hertzman PA, Clauw DJ, Kaufman LD, Varga J, Silver RM, Thacker HL, Mease P, Espinoza LR, Pincus T (1995) The eosinophilia-myalgia syndrome: status of 205 patients and results of treatment 2 years after onset. *Ann. Intern. Med.* **122**:851-855

9. Hibbs JR, Mittleman B, Hill P, Medsger TAJr (1992) L-tryptophan-associated eosinophilic fasciitis prior to the 1989 eosinophilia-myalgia syndrome outbreak. *Arthritis Rheum.* **35**:299-303

10. Shulman LE (1975) Diffuse fasciitis with eosinophilia: a new syndrome? *Trans. Assoc. Am. Physicians* **88**:70-86

11. Belongia EA, Hedberg CW, Gleich GJ, White KE, Mayeno AN, Loegering DA, Dunnette SL, Pirie PL, MacDonald KL, Osterholm MT (1990) An investigation of the cause of the eosinophilia-myalgia syndrome associated with tryptophan use. *N. Engl. J. Med.* **323**:357-365

12. Slutsker L, Hoesly FC, Miller L, Wailliams LP, Watson JC, Fleming DW (1990) Eosinophilia-myalgia syndrome associated with exposure to

tryptophan from a single manufacturer. *JAMA* **264**:213-217

13. Kamb ML, Murphy JJ, Jones JL, Caston JC, Nederlof K, Horney LF, Swygert LA, Falk H, Kilbourne EM (1992) Eosinophilia-myalgia syndrome in L-tryptophan-exposed patients. *JAMA* **267**:77-82

14. Jaffe RM (1989) Eosinophilia-myalgia syndrome caused by contaminated tryptophan. *Int. J. Biosocial Med. Research* **11**:181-184

15. Caston JC, Roufs JB, Forgarty CM, Applebaum ML, Smith WP Jr, Littlefield RH (1990) Treatment of refractory eosinophilia-myalgia syndrome associated with ingestion of L-tryptophan containing products. *Adv. Ther.* **7**:206-228

16. Hill RH Jr, Caudill SP, Philen RM, Bailey SL, Flanders WD, Driskell WJ, Kamb ML, Needham LL, Sampson EJ (1993) Contaminants in L-tryptophan associated with eosinophilia myalgia syndrome. *Arch. Environ. Contam. Toxicol.* **25**:134-142

17. Williamson BL, Johnson KL, Tomlinson AJ, Gleich GJ, Naylor S (1998) On-line HPLC-tandem mass spectrometry structural characterization of case-associated contaminants of L-tryptophan implicated with the onset of eosinophilia myalgia syndrome. *Toxicol. Letts.* **99**:139-150

18. Trulson ME, Sampson HW (1986) Ultrastructural changes of the liver following L-tryptophan ingestion in rats. *J. Nutr.* **116**:1109-1115

19. Shishikura T, Tsuchiya T, Sato F (1991) Eosinophilia caused by administration of L-tryptophan to animals with adrenal dysfunction. *Toxicol. Lett.* **58**:315-321

20. Stahl JL, Cook EB, Pariza MA, Cook ME, Graziano FM (2001) Effect of L-tryptophan supplementation on eosinophils and eotaxin in guinea pigs. *Exp. Biol. Med.* **226**:177-184

21. Crofford LJ, Rader JI, Dlakas MC, Hill RH Jr, Page SW, Needham LL, Brady LS, Heyes MP, Wilder RL, Gold PW, Illa I, Smith C, Sternberg EM (1990) L-tryptophan implicated in human eosinophilia-myalgia syndrome causes fasciitis and perimyositis in the Lewis rat. *J. Clin. Invest.* **86**:1757-1763

22. Love LA, Rader JI, Crofford LJ, Raybourne RB, Principato MA, Page SW, Trucksess MW, Smith MJ, Dugan EM, Turner ML, Zelazowski E, Zelazowski P, Sternberg EM (1993) Pathological and immunological effects of ingesting L-tryptophan and 1,1'-ethylidenebis(L-tryptophan) in Lewis rats. *J. Clin. Invest.* **91**:804-811

23. Silver RM, Ludwicka A, Hampton M, Ohba T, Bingel SA, Smith T, Harley RA, Maize J, Heyes MP (1994) A murine model of the eosinophilia-myalgia

syndrome induced by 1,1'-ethylidenebis(L-tryptophan). *J. Clin. Invest.* **93**:1473-1480

24. Emslie-Smith AM, Mayeno AN, Nakano S, Gleich GJ, Engel AG (1994) 1,1'-Ethylidenebis-[tryptophan] induces pathologic alterations in muscle similar to those observed in the eosinophilia-myalgia syndrome. *Neurology* **44**:2390-2392

25. Zangrilli JG, Mayeno AN, Vining V, Varga J (1995) 1,1'-ethylidenebis[L-tryptophan], an impurity in L-tryptophan associated with eosinophilia-myalgia syndrome, stimulates type 1 collagen gene expression in human fibroblasts *in vitro*. *Biochem. Mol. Biol. Int.* **37**:925-933

26. Takagi H, Ochoa MS, Zhou L, Helfman T, Murata H, Falanga V (1995) Enhanced collagen synthesis and transcription by peak E, a contaminant of L-tryptophan preparations associated with the eosinophilia-myalgia syndrome. *J. Clin. Invest.* **96**:2120-2125

27. Brenneman DE, Page SW, Schultzberg M, Thomas FS, Zelazowski P, Burnet P, Avidor R, Sternberg EM (1993) A decomposition product of a contaminant implicated in L-tryptophan eosinophilia myalgia syndrome affects spinal cord neuronal cell death and survival through stereospecific, maturation and partly interleukin-1-dependent mechanisms. *J. Pharmacol. Exp. Ther.* **266**:1029-1035

28. Yamaguchi Y, Tsunoda J, Suda T, Miura Y, Shiori-Nakano K, Kasahara T (1991) Effect of synthesized constituents in the L-tryptophan product on the differentiation of eosinophils and the induction of IL-6: a possible cause of Eosinophilia-Myalgia Syndrome. *Biochem. Biophy. Res. Comm.* **178**:1008-1013

29. Yamaoka KA, Miyasaka N, Inuo G, Saito I, Kolb J-P, Fujita K, Kashiwazaki S (1994) 1,1'-ethylidenebis(tryptophan) (peak E) induces functional activation of human eosinophils and interleukin 5 production from T lymphocytes: association of eosinophilia-myalgia syndrome with a L-tryptophan contaminant. *J. Clin. Immunol.* **14**:50-60

30. Mayeno AN, Benson LM, Naylor S, Colberg-Beers M, Puchalski JT, Gleich GJ (1995) Biotransformation of 3-(phenylamino)-1,2-propanediol to 3-(phenylamino)alanine: a chemical link between toxic oil syndrome and eosinophilia-myalgia syndrome. *Chem. Res. Toxicol.* **8**:911-916

31. Sato F, Hagiwara Y, Kawase Y (1995) Subchronic toxicity of 3-phenylamino alanine, an impurity in L-tryptophan reported to be associated with eosinophilia-myalgia syndrome. *Arch. Toxicol.* **69**:444-449

32. Sidransky H, Verney E, Cosgrove JW, Latham PS (1994) Effect of 3-phenylamino-L-alanine on tryptophan binding to rat hepatic nuclear envelopes. *Toxicology* **86**:135-145

33. Flockhart DA, Clauw DJ, Sale EB, Hewett J, Woosley RL (1994) Pharmacogenetic characteristics of the eosinophilia-myalgia syndrome. *Clin. Pharmacol. Ther.* **56**:398-405

34. Spitzer WO, Haggerty JL, Berkson L, Davis W, Palmer W, Tamblyn R, Laprise R, Faith JM, Elmore JG, Horwitz RI (1995) Continuing occurrence of eosinophilia myalgia syndrome in Canada. *Br. J. Rheumatol.* **34**:246-251

35. Clauw DJ, Flockhart DA, Mullins W, Katz P, Medsger TA Jr (1994) Eosinophilia-myalgia syndrome not associated with the ingestion of nutritional supplements. *J. Rheumatol.* **21**:2385-2387

36. Margolin L (2003) Non-L-tryptophan related eosinophilia-myalgia syndrome with hypoproteinemia and hypoalbuminemia. *J. Rheumatol.* **30**:628-629

37. Sternberg EM, van Woert MH, Young SN, Magnussen I, Baker H, Gauthier S, Osterland CK (1980) Development of a scleroderma-like illness during therapy with L-5-hydroxytryptophan and carbidopa. *N. Engl. J. Med.* **303**:782-787

38. Farinelli S, Mariani A, Grimaldi A, Mariani M, Iannessi A, De Rosa F (1991) Sindrome eosinofilia-mialgia associata a 5-OH-triptofano. Descrizione di un caso. *Recenti Prog. Med.* **82**:381-384

39. Michelson D, Page SW, Casey R, Trucksess MW, Love LA, Milstien S, Wilson C, Massaquoi SG, Crofford LJ, Hallett M, Gold PW, Sternberg EM (1994) An eosinophilia-myalgia syndrome related disorder associated with exposure to L-5-hydroxytryptophan. *J. Rheumatol.* **21**:2261-2264

40. Williamson BL, Klarskov K, Tomlinson AJ, Gleich GJ, Naylor S (1998) Problems with over-the-counter 5-hydroxy-L-tryptophan. *Nature Medicine* **4**:983

41. Waller P, Wood S, Breckenridge A, Rawlins M (1991) Eosinophilia-myalgia syndrome associated with prescribed L-tryptophan in the United Kingdom. *Health Trends* **23**:53-55

42. Merck Pharmaceuticals (2003) Optimax/L-tryptophan assessment. Submitted to the COT March 2004.

43. NPN (2003) Personal communication from the Dutch trade association Natuur- en gezondheids Producten Nederland to the COT Secretariat, 4 November 2003, by email.

44. HFMA (2003). Personal communication from the Health Food Manufacturers Association to the Food Standards Agency, 27 August

2003, by email.

45. Health Council of the Netherlands (1999) Safety of amino acid supplementation. Committee on amino acid supplementation. Publication no. 1999/06. The Hague.